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"Effects of High and Low Barometric Pressures on Susceptibility and Resistance to Infection"

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Abstract. Observations are reported on the fecal flora of mice observed over a period of 16 weeks, the parabaric groups being maintained in decreased and increased pressures but with a normal pO_2. A transient increase in Klebsiella was seen in the two parabaric groups compared to controls, and a more sustained increase in a Streptococcus.

Our first experiment using Salmonella typhimurium as a challenge agent in parabaric mice (increased O_2) is reported. Borderline differences were seen between control and test groups; the experiments must be repeated before an interpretation is possible.

Experiments completed to date, using the mouse pneumonitis agent (Chlamydia) as a respiratory tract challenge pathogen, are summarized. The stress of varied pressure appears to affect this model infection; increased O_2 may have a direct adverse effect on growth of the agent.

During several simulated group dives at the Naval Experimental Diving Unit the microflora from 5 sites of the personnel involved were assayed, as well as specimens from the environment. These tests serve as guides for planning further more extensive tests of this nature in actual or simulated dives.

Progress has been quite satisfactory in study of interferon production under parabaric conditions at both the cellular and the animal level. Definite effects of alterations in O_2 were observed in cells in vitro, and in lung tissue of mice.

A noteworthy difference was seen in the effect of O_2 on metabolism in 2 strains of Clostridium perfringens, one pathogenic, and one nonpathogenic. Only the pyruvic oxidase activity of the former was reduced by O_2.

1. Effect of parabarosis on enteric bacterial flora of mice. (F. B. Gordon and J. D. Gillmore)

In the preceding quarterly report Exp. 9 was mentioned, the first in a series designed to determine the effect of hyperbaric and hypobaric conditions, with normal pO_2, on enteric bacterial flora of the mouse. Exp. 9 is now completed and significant data are presented in Figs 1, 2, and 3. Thirty mice, beginning at 3 weeks of age, were kept together for 3 weeks to promote a homogenous enteric flora and were then (time 0) divided into 3 groups, each of which was placed in a similar type of plexiglass chamber. The environmental conditions for each group were as follows, all representing 160 mm of O_2 (normal sea level tension):

Normobaric group: air from compressed air line, at one atmosphere.

Hypobaric group: 100% O_2 at 3 psia; simulated 37,000 ft. altitude.

Hyperbaric group: 2.8% O_2 at 110 psia; simulated 213 ft. depth in sea water.

The chambers were brought to normal pressures once a week, opened, and cleaned. The mice were weighed, fed and watered, and every two weeks a fecal pellet was cultured in the same manner as has been described in previous reports. The plan of the experiment called for return of all 3 groups to the shelf at 12 weeks, with one or more subsequent stool cultures. Unfortunately, the mice of the normobaric and hypobaric groups were lost between the 10th and 12th weeks due to mechanical or operating failures, and only the hyperbaric group could be followed subsequent to the return to normal conditions.

With respect to average weight curves for the 3 groups, Fig. 1, it is apparent that increased pressure adversely affected weight gain, the average weight of the hyperbaric group being about 6 gms less than the normobaric group at 4 weeks after placing the mice in the chambers (i.e., at 10 weeks of age). The weight curve suggests that a turn toward normal may have begun between the 10th and 11th weeks, but a rapid rise followed return to normal shelf conditions at 12 weeks. By comparison with previously obtained weight data on normal shelf mice it is apparent that some depression of expected weight gain occurred in the other 2 groups of mice as well.

Of particular interest was the incidence of Klebsiella in these mice because of the differences seen in previous experiments, with respect to this type of bacterium. The two parabaric groups of mice showed a distinct rise in Klebsiella incidence, as compared with the normal (Fig. 2), at the 4th week after exposure to parabaric conditions, but this increase was not sustained as was seen in groups of mice exposed to increased O_2 tensions in previous experiments. It is quite possible that the shifts in Klebsiella incidence in the two types of experiments are a response to some factor such as stress, per se, rather than a direct effect of a specific element of the parabaric environment.

A second significant difference between normobaric and hyperbaric mice in this experiment was seen in the incidence (Fig. 3) of a streptococcus (facultative anaerobe appearing on Schaedler B medium). In the hypobaric mice the incidence of this species took an intermediate position.

Other bacterial types cultured showed no noteworthy differences in incidence among the 3 groups. These types were: slow lactose fermenters, a second coliform type, 2 colonial types of anaerobic lactobacillus, and 3 types of Bacteroides.

Further experiments in this series were delayed due to necessary repairs, retesting and renovation of the animal chambers used. This has now been accomplished, along with the acquisition of 3 additional chambers of the same type.

2. Effect of parabarosis on pulmonary infection of mice with a chlamydial agent (mouse pneumonitis). (F. B. Gordon and J. D. Gillmore)

The investigations completed in this series are summarized in Table 1. They consist of 3 experiments in which increased O_2 (77%) at one atmosphere was used with 2 types of controls, and one in which normobaric, hypobaric, and hyperbaric conditions were employed, as described in Section 1 of this report. All mice were exposed for 15 min. to an infectious aerosol in a modified Henderson apparatus, providing for inhalation of approximately 7000 infective units per mouse. This led to progressive lung involvement and consolidation by the 5th-7th day, and reaching a maximum just short of death on the 9th day, when the mice were sacrificed. After the degree of gross pathology of the lungs was estimated, the smears made to confirm the specificity of the lesions, the lungs were pooled, emulsified, and stored for eventual titration by counting the number of intracellular inclusions produced in infected cell monolayer cultures.

Interpretation of the first 3 experiments listed, those involving 77% O_2, is as follows: No real differences were observed between the two types of controls, in room air on the shelf, and in chambers supplied by tank air. Exposure to O_2 during two weeks prior to infection by aerosol, and air afterwards, may have had some depressive effect on the borderline of significance. Exposure to O_2 only after infection, and both before and after infection resulted in a marked depression of the infection, as indicated by several criteria. These include the actual degree of growth of the chlamydial agent in the lung, and conform to some preliminary experiments, to be reported later, on growth of a similar agent in cell culture at different levels of O_2.

The fourth experiment shown in Table 1 indicates that mice under hypobaric conditions, but in normal pO_2, respond to the infection in much the same manner as the controls. They presented perhaps a poorer general appearance. In contrast, the group in a hyperbaric environment, but with normal pO_2 appeared much sicker and had a higher level of infectious agent in their lungs. In spite of this the pneumonic process was less, as indicated by lung weight and gross pathology. These findings are not inconsistent with what might be expected with infection in a stressed animal. The weights of the mice in this experiment, as well as those depicted in Fig. 1 for mice under similar conditions in another experiment indicate that some factor in the hyperbaric environment has a profound adverse effect on the mice.

The similar levels of chlamydial agent revealed by titrations of the control lungs in the different experiments indicate that this method of infection with

the mouse pneumonitis agent provides a regular, predictable, and reproducible effect.

3. Effect of increased O_2 on susceptibility of mice to Salmonella typhimurium. (LTJG D.G. Martin)

In previous experiments of this type we used the more acute infection of mice with S. typhi, but found no clear-cut differences in mortality between groups exposed to increased O_2 (77%) and the controls. Attention has now turned to a more chronic type of infection induced by S. typhimurium. A total of 75 mice were injected intraperitoneally with a saline suspension of S. typhimurium (approx. 3×10^3 cells/mouse). Approximately 1/3 of the mice were placed in 77% O_2, 1/3 in flowing air, and 1/3 on the shelf. The mice were observed and the number of deaths recorded daily. At the end of one week 5 mice from each group were sacrificed and the spleens cultured. The remaining mice were left in the environments for an additional week and the surviving mice at the end of this time were sacrificed and the spleens cultured.

All spleen cultures, at both one and two weeks, were positive for S. typhimurium. Quantitative cultures were not made.

Table 2 summarizes the results and indicates that the mortality figures for each group are similar at the end of 2 weeks. At one week, however, there was possibly a significant difference between mice housed in chambers, in flowing gas (77% O_2 and air from tank), and those in room air on the shelf. In comparing the O_2 and room air groups the significance is estimated to lie between 5 and 10% ($X^2 = 2.78$). This result will have to be confirmed before it can be considered noteworthy.

4. Observations on microflora of divers. (CAPT C. E. Meyers and Mr. J. D. Gillmore)

With the excellent cooperation of the staff of the Experimental Diving Unit (EDU), U. S. Navy, it has been possible to sample divers and their environment in simulated saturation dives involving continued confinement and increased pressure over periods of days. Assay of microbiological samples from the four subjects in the first EDU trial have been partially completed. The trial lasted nine days, including decompression time. The chamber pressure was equivalent to a depth of 450 feet and one-hour excursions to 600 feet were made by divers in pairs using scuba gear.* The design of the chamber permits the 600 feet excursion divers to enter the separately pressurized lower half of the chamber (the "wet-pot") and perform various tasks in approximately eight feet of water. The upper half of the chamber, sealed with an appropriate hatch, remains at 450 feet. The atmosphere breathed was oxygen and helium, the temperature was approximately 85 F, and the RH ranged from 65 to 79%.

Samples were collected daily from five body areas: nasal cavity, oropharynx, skin, ear, and feces. (Feces were collected according to the subjects' individual bowel movement habits). All samples were collected by swabbing the areas using a definite procedure with swabs moistened and stored in skimmed milk.

* Pressures, depths, and other details of these dives are a personal communication from the Medical Officer of EDU, and until published, must be regarded as privileged information for official use only.

Samples were taken immediately prior to transfer of food into the chamber, passed out through a lock with return of food, and immediately stored at -60 C until assay. Preliminary studies upon loss due to freezing indicated approximately a one to two log loss, but with apparently little selective losses among the microflora obtained from laboratory personnel. Samples also were collected from the water of the wet-pot, and in subsequent trials from the atmosphere.

Briefly, the findings to date are:

1. Enteric microorganisms were recovered from the wet-pot in high concentrations, and from the throats of two of the four subjects;

2. Immediately after each excursion dive, the skin mannite positive staphylococci no longer were recovered, although the total staphylococcus count remained essentially the same. Twenty-four through 72 hours following the 600-foot excursion, the mannite positive staphylococci reappeared in increased numbers in all subjects and in addition appeared in sampling areas where they were not present in base line counts. A penicillin resistant and optochin sensitive strain morphologically resembling pneumococcus was first isolated from the throat of two of the subjects and later from the throat and nose of one of the two on the third day. Although the organism was not bile-soluble, its appearance was associated with clinical symptoms of sore throat, etc. Further tests to identify the organism are in progress.

It is difficult to evaluate the information at present, before the data are complete and the three subsequent trials are assayed. Obviously, the presence of enteric organisms in the throats of some of the subjects is not desirable, but beyond this, the data are too preliminary to allow even speculation.

5. Effect of parabarosis on interferon production. (Dr. K-Y. Huang)

Observations on the effect of parabarosis on interferon production have been extended in a series of experiments involving both intact animals (mice) and cultivated cells (mouse fibroblasts). The range of environmental conditions has been broadened to include simulated altitude and depth, as well as hyperoxia and hypoxia. Newcastle disease virus (NDV) and statolon, a non-viral interferon inducer, were used as the main inducers of interferon.

Experiment 5. In the last report, results were presented which indicated that hyperoxia did not affect the level of serum interferon in mice to any significant extent. This experiment (5) was carried out to observe the effect of hypoxia. Two groups of mice were kept, respectively, in a chamber of 11% O_2 in N_2 (pO_2 equivalent to that at an altitude of approximately 17,500 ft.), and the other in air from a tank. After three days each mouse was injected with 0.4 ml of NDV in chick allantoic fluid containing 6×10^7 plaque-forming units (pfu). Groups of 7 or 10 mice from test and control groups were sacrificed and sera and lungs were collected at intervals of 2, 3, 10 and 24 hours after injection. The pooled serum specimens and extracts of lungs were processed for interferon assay by the standard technique. Both groups gave similar curves (Fig. 4) of interferon levels both in sera and lungs; differences between the two groups were insignificant. It must be pointed out, however, that the lungs

unlike those in Experiment 6 (see below), were not perfused. The difference in levels of lung interferons might have been obscured by the relatively large quantity of blood remaining in non-perfused lungs.

Experiment 6. Two groups of mice were kept, respectively, in a chamber maintained at 1 atmosphere with 11% O_2 in N_2, and the other in air from a tank. After six days, each mouse was injected with NDV as in Exp. 5. At intervals of 4, 10, and 24 hours, groups of 5 mice from test and control groups were sacrificed. The lungs were immediately perfused with 0.85% NaCl, removed, extracted with Eagle's medium and processed for interferon assay. Fig. 5 shows the results of this experiment. Interferon level tends to be higher in the hypoxic lungs than in control lungs. The difference is especially marked, and significant, 24 hours after interferon induction, suggesting that interferon level might be maintained longer in hypoxic lungs than in control lungs. Experiments have been planned to extend the observation beyond 24 hours.

Experiment 7. The effect of altered barometric pressures on induction of interferon was studied. Three groups of mice were maintained respectively at simulated depth (213 ft. in sea water), simulated altitude (37,000 ft.), and at sea level, all at normal pO_2 in N_2, alone or in air. After two weeks, all mice were induced with NDV as in the previous two experiments. Groups of 4 or 5 mice from each test and control group were bled at intervals of 2, 10, and 24 hours and pooled sera were assayed for interferon. In mice exposed to increased or decreased pressures, the interferon levels in sera were significantly lower than in the control group (Fig. 6). The overall pattern of the curves, however, were similar, suggesting a general depression in ability to produce interferon under increased or decreased barometric pressures. The possibility of stress as the cause of the depression in interferon level cannot be ruled out.

Experiments at cellular levels have also been carried out. L cells were used for this purpose. To create parabaric conditions, tubes of cultivated cells were either individually gassed or placed in a chamber which was gassed with desired mixture of gases.

Experiment C-3. Tubes of cells were incubated overnight in air. They were then induced for interferon by one of the following three inducers: (1) statolon, 100 µg; (2) statolon, 150 µg; (3) Sindbis virus, 10^7 pfu. Tubes receiving the same inducers were divided into two groups and were incubated respectively in 72% O_2 or in air. Culture fluids were harvested after overnight incubation and assayed for interferon. As shown in Table 3, increased O_2 depressed the induction of interferon by 100 µg statolon and Sindbis virus. The induction by 150 µg of statolon, however, was not affected.

Experiment C-4. Interferon was induced in 95% O_2 by different doses of inducer. The depressive effect of oxygen on the induction of interferon was more marked than that observed in Experiment C-3. Such a depressive effect was also observed in the induction by 150 µg statolon (Table 4).

Experiment C-5. Cells were cultivated first in air or in 72% O_2, then were induced by 100 µg statolon, and incubation was continued in 72% O_2 or in air. The results shown in Table 5 clearly indicate that cells exposed to increased O_2 before but not during induction showed the same degree of depressed inter-

feron production as those which were induced under O_2 or those which were exposed before and during induction. Apparently the inhibitory effect of O_2 can persist for a certain period of time after return to a normal gaseous environment.

The depression of interferon production in cultivated cells by O_2 is in line with the finding of increased interferon in hypoxic lungs. The difference seen in in vitro experiments cannot be attributed to a difference in the number of cells available for producing interferon, since cells incubated 48 hours in 72% or 95% O_2 gave the same count as those incubated in air. Further study is planned to obtain more insight into whether the depressive effect of oxygen on interferon production is a specific one or is merely the expression of a general damaging effect of O_2 on cellular metabolism, such as protein synthesis or energy production.

6. Studies on altered gaseous environment at the cellular level. (Drs. E. M. Neptune, Jr. and E. Weiss)

Previous reports have shown that microbial enzymes are very sensitive to the gaseous environment to which they are exposed. In Chlamydia the pentose phosphate pathway, the pyruvic oxidase, and α-ketoglutarate dehydrogenase systems are all responsive in various ways to the gas environment. Details of these studies have been included in earlier reports.

Currently studies are being performed with Clostridium perfringens. With respect to growth characteristics this organism is a classical obligate anaerobe. Preliminary studies have been performed with a strain that is highly pathogenic, as evidenced by production of gas gangrene in dogs in previous unrelated studies. The pyruvic oxidase enzyme of this microbe is very active in a nitrogen environment and markedly suppressed by air or oxygen. This enzyme assay extends over a period of 1 to 2 hours. Rather surprisingly, when a nonpathogenic strain of the same microbe was studied, its pyruvic oxidase activity showed no difference in nitrogen, air or oxygen. These and other enzyme studies are being extended to investigate further the relationship, if any, between pathogenicity and enzymatic activity.

Table 1. Effect of parabaric conditions on mouse lung infection with a chlamydial agent (mouse pneumonitis).

Environment	Time of exposure	D/T	Appearance on day 9	Av. mouse wt.	Av. lung wt.	Gross pathology of lung	Lung titer IFU/ml, X 10^5
77% O_2	2 weeks before infection	1/10	active,ruffled	13.9	0.63	2.7	28 ± 4
Tank air		1/10	lethargic,ill	15.2	0.62	3.8	47 ± 1
Room		1/10	" "	15.8	0.55	3.1	51 ± 7
77% O_2	9 days after infection	0/10	normal	15.9	0.25	0.8	< *
Tank air		0/10	lethargic,ill	16.3	0.47	2.0	>
Room		0/10	active,ruffled	16.8	0.49	2.3	–
77% O_2	2 weeks before and 9 days after	0/11	normal	18.8	0.24	2.4 **	2.6 ± 0.6
Tank air		0/11	active,ruffled	15.1	0.56	1.6	32 ± 2
Room		0/11	lethargic,ill	14.5	0.71	1.5	50 ± 5
2.8% O_2 at 110 psia***	2 wks before & 9 days after	0/10	lethargic,ill	11.8	0.26	1.1	184 ± 44
100% O_2 at 3 psia		0/7	active,ruffled	15.0	0.48	1.3	67 ± 13
Air from tank 14.7 psia		0/10	normal	16.2	0.45	1.7	56 ± 5

* Titration unsatisfactory. It was possible only to estimate roughly the relative levels of Chlamydia in two groups.

** Inclusion bodies not found in lung impression smears; interpretation doubtful.

*** In each group of this experiment the pO_2 equalled 160 mm Hg, i.e., normal.

Table 2. Effect of altered environments on infection of mice with Salmonella typhimurium.

Interval	Cumulative mortality (%) in three environments after inoculation		
	77% O_2	Air from tank	Air of room
7 days	21	28	43
14 days *	71	68	78

* Cumulative figures corrected for removal of 5 mice from each group for culture at 7 days.

Table 3. Induction of interferon in L cells under 72% O_2 and under air.

Inducer	Interferon titer (units/ml)	
	72% O_2	Air
Statolon, 100 µg	316	1,258
" 150 µg	1,000	1,000
Sindbis virus	5	32
Control	0	0

Table 4. Induction of interferon in L cells under 95% O_2 and under air.

Dose of statolon	Interferon titer (units/ml)	
	95% O_2	Air
10 µg	0	10
50 µg	5	63
100 µg	19	80
150 µg	39	126
Control	0	0

Table 5. Induction of interferon in L cells by 100 µg/ml statolon; under 72% O_2 and under air.

Gas phase		Titer of interferon
Before induction	During induction	(units/ml)
air	air	208
air	O_2	39
O_2	air	30
O_2	O_2	79

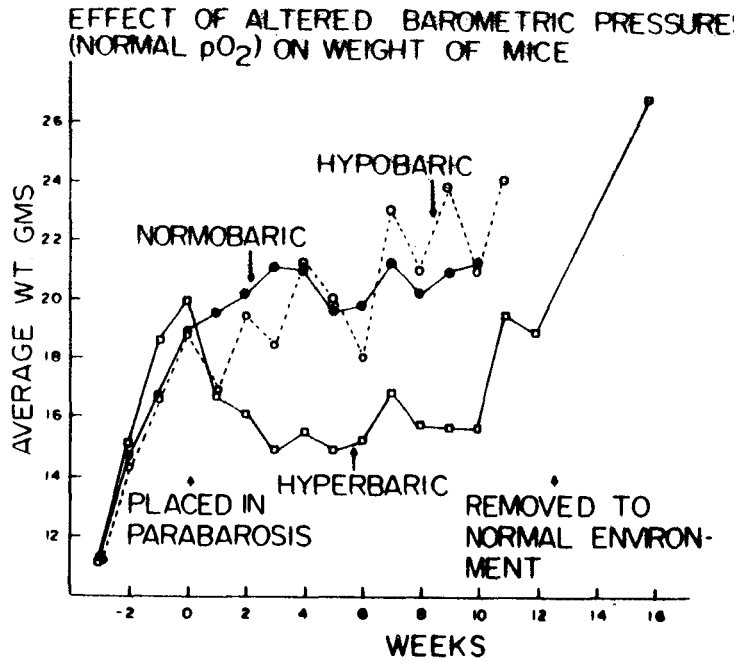

Fig. 1. Average weights of mice held in hypobaric (3 psia; simulated 37,000 ft. altitude), hyperbaric (110 psia; simulated 213 ft. depth in the sea), and normobaric (air at one atmosphere pressure) environments. All groups were in an atmosphere containing 160 mm Hg of O_2 (normal at sea level).

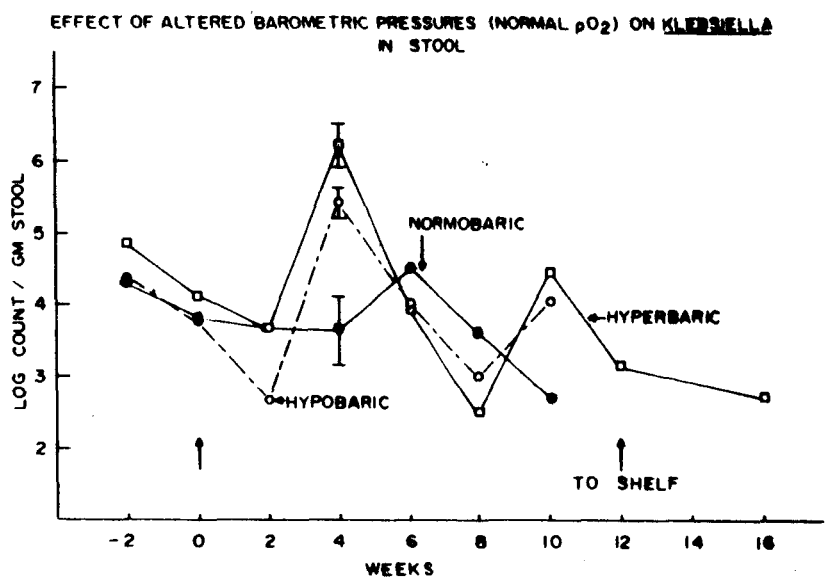

Fig. 2. Average numbers of Klebsiella in stools of mice kept in the 3 environments described in caption of Fig. 1.

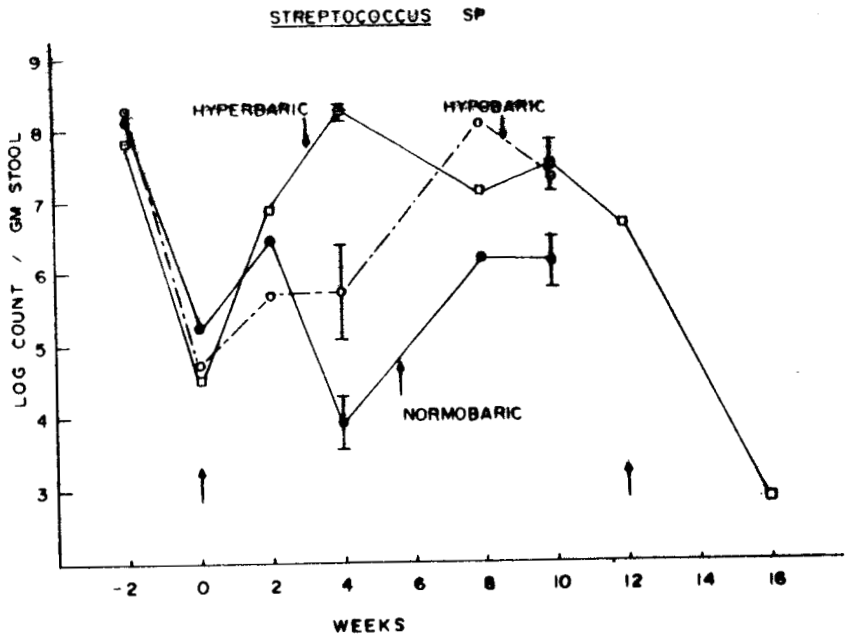

Fig. 3. Average numbers of Streptococcus sp. in stools of mice kept in the 3 environments described in caption of Fig. 1.

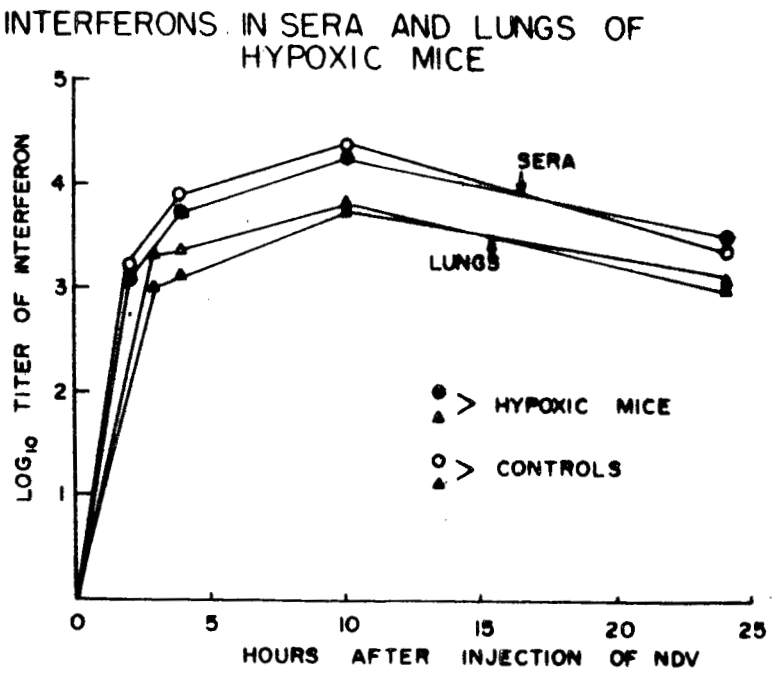

Fig. 4. Levels of interferon in the sera and lungs of mice after maintenance for 3 days in decreased pO_2 (11%), and in air (controls). Induction of interferon was by intravenous inoculation of Newcastle disease virus.

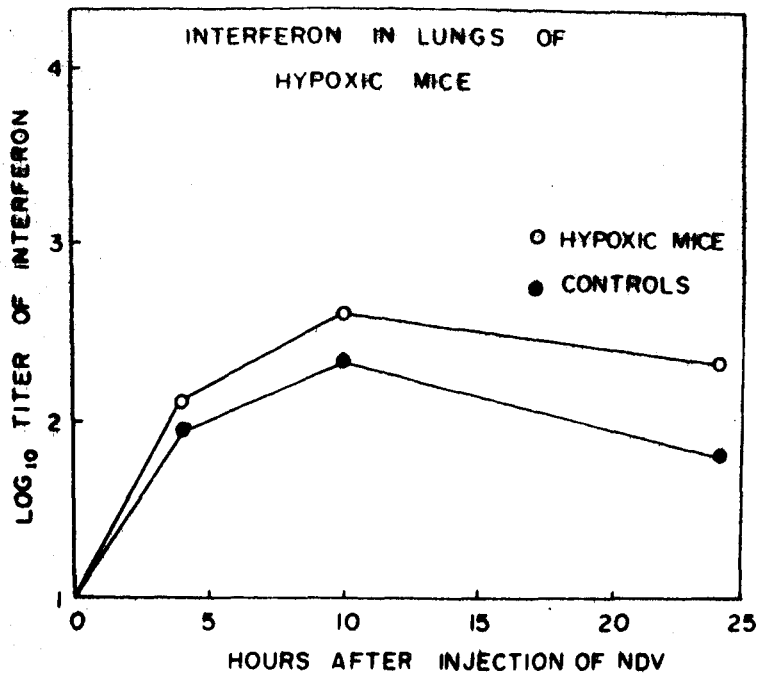

Fig. 5. Levels of interferon in the lungs of mice after maintenance for 3 days in decreased pO_2 (11%). Induction was by intravenous injection of Newcastle disease virus. Lungs were perfused to remove the blood.

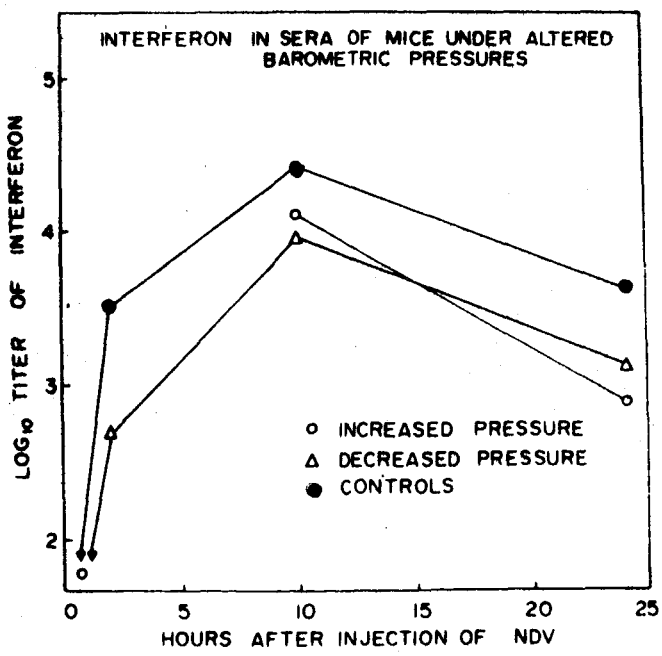

Fig. 6. Levels of interferon in the sera of mice maintained at simulated depth (213 ft. in sea water, o), simulated altitude (37,000 ft., Δ), and at sea level (●), all at normal pO_2 in N_2, alone, or in air. Interferon was induced by the injection of Newcastle disease virus.